Claims

We claim:

1. A method to detect a first transgenic nucleic acid molecule in a sample having either (a) a detectable amount of both a first and a second transgenic nucleic acid molecule or (b) a substantially non-detectable amount of said molecules, said method comprising hybridizing said second transgenic nucleic acid molecule in said sample with at least one oligonucleotide designed to hybridize to said second transgenic nucleic acid molecule, whereby said hybridizing indicates the presence of said first transgenic nucleic acid molecule in the sample .

2. A method according to claim 1 further comprising quantitation of said second transgenic nucleic acid molecule.

3. A method according to claim 1 wherein the at least one oligonucleotide hybridizes to said second transgenic nucleic acid molecule selected from the group consisting of marker sequences, vector backbone sequences, promoter sequences, signal sequences, 3' UTR sequences, 5' UTR sequences, tDNA border sequences, and enhancer sequences.

4. A method according to claim 1 wherein the at least one oligonucleotide hybridizes to a second transgenic nucleic acid molecule selected from the group consisting of a 35S cauliflower mosaic virus promoter, a NOS promoter, an Adh promoter, a NPTII gene, an ampicillan resistance gene, a chloramphenical-resistance gene, a tDNA left border sequence, a tDNA right border sequence, a Petunia HSP70 5' untranslated leader sequence, a wheat fructose 1,6-biphosphatase 5' untranslated leader, a 3'untranslated sequence from the 3' end of the *Pisum sativum* rbcS E9 gene, a 3' untranslated sequence from the wheat ubiquitin gene and a 3' untranslated sequence from the nopaline synthase gene.

5. A method according to claim 1 wherein the at least one oligonucleotide hybridizes to a second transgenic nucleic acid molecule having a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO: 6 and SEQ ID 29 to SEQ ID 35.

6. A method according to claim 1 wherein the at least one oligonucleotide is a sequence selected from the group consisting of SEQ ID NO:7 to SEQ ID NO:28.

7. A method according to claim 1 further comprising amplification of said second transgenic nucleic acid molecule.

8. A method according to claim 7 further comprising quantitation of said second transgenic nucleic acid molecule.

9. A method according to claim 7 wherein the amplification is carried out by a method selected from the group consisting of PCR or RT-PCR.

10. A method according to claim 7 wherein the quantitation of said second transgenic nucleic acid molecule is determined by a method selected from the group consisting of quantitative PCR, quantitative RT-PCR, competitive quantitative PCR or competitive quantitative RT-PCR.

11. A method according to claim 7 wherein said second transgenic nucleic acid nucleic molecule is derived from a nucleic acid molecule selected from the group consisting of a 35S caluiflower mosaic virus, a NOS promoter, an Adh promoter, a NPTII gene, an ampicillan resistance gene, a chloramphenical-resistance gene, a tDNA left border sequence, a tDNA right border sequence, a Petunia HSP70 5' untranslated leader sequence, a wheat fructose 1,6-biphosphatase 5' untranslated leader, a 3' untranslated sequence from the 3' end of the *Pisum sativum* rbcS E9 gene, a 3' untranslated sequence from the wheat ubiquitin gene and a 3' untranslated sequence from the nopaline synthase gene.

12. A method according to claim 11 wherein said second transgenic nucleic acid molecule comprises at least 100 base pairs of consecutive sequence having substantial identity to

a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:6 and SEQ ID 29 to SEQ ID 35.

13. A method according to claim 12 wherein said at least one oligonucleotide comprises at least 15 bases substantially identical or complementary to a consecutive sequence of a second transgenic nucleic acid molecule having a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:6 and SEQ ID NO: 29 to SEQ ID NO: 35.

14. A method according to claim 13 wherein said at least one oligonucleotide has a sequence selected from the group consisting of SEQ ID NO: 7 through SEQ ID NO: 28.

15. A method according to claim 14 wherein said at least one oligonucletide has a detectable label.

16. A method according to claim 15 wherein said label is selected from the group consisting of a fluorescent label, a digoxigenen-dUTP label, a biotin label, and a radiolabel.

17. A method according to claim 7 wherein said at least one oligonucleotide comprises a primer pair and a probe designed to hybridize to said second transgenic nucleic acid molecule in a 5' nuclease assay.

18. A method according to claim 17 wherein each of said primer pair used in said amplification comprises 15 to 30 bases identical or complementary to a consecutive sequence of a second transgenic nucleic acid molecule said second transgenic nucleic acid molecule molecule having a sequence selected from the group consisting of marker sequences, vector backbone sequences promoter sequences, signal sequences, 3' UTR sequences, 5' UTR sequences, tDNA border sequences, and enhancer sequences and wherein said probe comprises 15 to 30 bases complementary or identical to a second transgenic nucleic acid molecule having a sequence selected from the group consisting of marker sequences, signal sequences, vector backbone sequences promoter sequences, 3' UTR sequences, 5' UTR sequences, tDNA border sequences, and enhancer sequences.

19. A method according to claim 17 wherein each of said primer pair used in said amplification comprises 15 to 30 bases identical or complementary to a consecutive sequence of a second transgenic nucleic acid molecule said second transgenic nucleic acid molecule molecule having a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:6 and SEQ ID NO: 29 to SEQ ID NO: 35 and wherein said probe comprises 15 to 30 bases identical or complementary to a second transgenic nucleic acid molecule having a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:6 and SEQ ID NO: 29 to SEQ ID NO: 35.

20. The method according to claim 17 wherein said primer pair and said probe used for the amplification process are selected from the group consisting of Seq ID NO:7 to Seq ID NO:8 with the probe SEQ ID NO:9, SEQ ID NO: 7 and SEQ ID NO: 28 with the probe SEQ ID NO: 9, SEQ ID NO: 10 to SEQ ID NO: 11 with the probe SEQ ID NO: 12, SEQ ID NO: 13 to SEQ ID NO: 14 with the probe SEQ ID NO: 15, SEQ ID NO: 16 to SEQ ID NO: 17 with the probe SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID NO: 20 with the probe SEQ ID NO: 21, SEQ ID NO: 19 and SEQ ID NO: 20 with the probe SEQ ID NO: 22, and SEQ ID NO: 23 to SEQ ID NO: 24 with the probe SEQ ID NO: 25.

21 A method according to claim 1 further comprising Southern Blotting, Northern Blotting or RNAse protection assay.

22 A method according to claim 21 further comprising quantitation of said second transgenic nucleic acid molecule.

23 A method according to claim 21 wherein said at least one oligonucleotide is a probe which hybridizes to a second transgenic nucleic acid molecule selected from the group consisting of marker sequences, vector backbone sequences, signal sequences, promoter sequences, 3' UTR sequences, 5' UTR sequences, tDNA border sequences, and enhancer sequences.

24 A method according to claim 21 wherein said probe hybridizes to a second transgenic nucleic acid molecule selected from the group consisting of a 35S cauliflower mosaic virus, a

NOS promoter, an Adh promoter, a NPTII gene, an ampicillan resistance gene, a chloramphenical-resistance gene, a tDNA left border sequence, a tDNA right border sequence, a Petunia HSP70 5' untranslated leader sequence, a wheat fructose 1,6-biphosphatase 5' untranslated leader, a 3'untranslated sequence from the 3' end of the *Pisum sativum* rbcS E9 gene, a 3' untranslated sequence from the wheat ubiquitin gene and a 3' untranslated sequence from the nopaline synthase gene.

25. A method according to claim 21 wherein said probe hybridizes to a second transgenic nucleic acid molecule having a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:6 and SEQ ID NO: 29 to SEQ ID NO: 35.

26. A method according to claim 21 wherein said probe is a sequence selected from the group consisting of SEQ ID 7 to SEQ ID 28.

27. A method according to claim 25 wherein said probe has a detectable label.

28. A method according to claim 26 wherein said label is selected from the group consisting of a fluorescent label, a digoxigenen-dUTP label, a biotin label, and a radiolabel.

29. An amplification kit for the detection of a second transgenic nucleic acid molecule comprising at least one primer pair and a corresponding labeled probe which hybridizes to a promoter selected from the group consisting of 35S caluiflower mosaic virus, a NOS promoter and Adh promoter, a marker selected from the group consisting of NPTII gene, an ampicillan resistance gene and a chloramphenical-resistance gene, a plasmid sequence selected from the group consisting of tDNA left border sequence and tDNA right border sequence, a 5' untranslated region selected from the group consisting of Petunia HSP70 5' untranslated leader sequence and wheat fructose 1,6-biphosphatase 5' untranslated leader, a 3'untranslated sequence selected from the group of a 3' end of the *Pisum sativum* rbcS E9 gene, a 3' untranslated sequence from the wheat ubiquitin gene and a 3' untranslated sequence from the nopaline synthase gene.

30. A kit according to claim 28 wherein said primer pair and said corresponding labeled probe have nucleic acid sequence selected from the group consisting of promoter sequences SEQ ID NO: 1, SEQ ID NO: 6, SEQ ID NO: 31, marker sequences SEQ ID NO: 3, SEQ ID NO: 29, SEQ ID NO: 30, tDNA plasmid sequences SEQ ID NO: 4, SEQ ID NO: 34; 3' UTR sequences SEQ ID NO: 2, SEQ ID NO: 32, 5' UTR sequences SEQ ID NO: 5, SEQ ID NO: 33, SEQ ID NO: 35.

31. A kit according to claim 28 wherein said primer pair and said corresponding labled probe is selected from the group consisting of Seq ID NO:7 to Seq ID NO:8 with the probe SEQ ID NO:9, SEQ ID NO: 7 and SEQ ID NO: 28 with the probe SEQ ID NO: 9, SEQ ID NO: 10 to SEQ ID NO: 11 with the probe SEQ ID NO: 12, SEQ ID NO: 13 to SEQ ID NO: 14 with the probe SEQ ID NO: 15, SEQ ID NO: 16 to SEQ ID NO: 17 with the probe SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID NO: 20 with the probe SEQ ID NO: 21, SEQ ID NO: 19 and SEQ ID NO: 20 with the probe SEQ ID NO: 22 and SEQ ID NO: 23 to SEQ ID NO: 24 with the probe SEQ ID NO: 25.

32. A kit according to claim 28 further comprising quantitation of said second transgenic nucleic acid molecule.

33. A method to detect expression of a first transgenic nucleic acid molecule in a sample having either (a) a detectable amount of both a first and a second transgenic nucleic acid molecule or (b) a substantially non-detectable amount of said molecules, said method comprising hybridizing said second transgenic nucleic acid molecule in said sample with at least one oligonucleotide designed to hybridize to said second transgenic nucleic acid molecule, whereby said hybridizing indicates the presence of said first transgenic nucleic acid molecule in the sample and said at least one oligonucleotide hybridizes to a 3' untranslated region further comprising quantitative PCR. .

34. A method according to claim 32 wherein said oligonucleotide hybridizes to SEQ ID NO: 5.

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